

Figure 2: 2'-O-Me substituted Amberzyme Enzymatic Nucleic Acid Motif

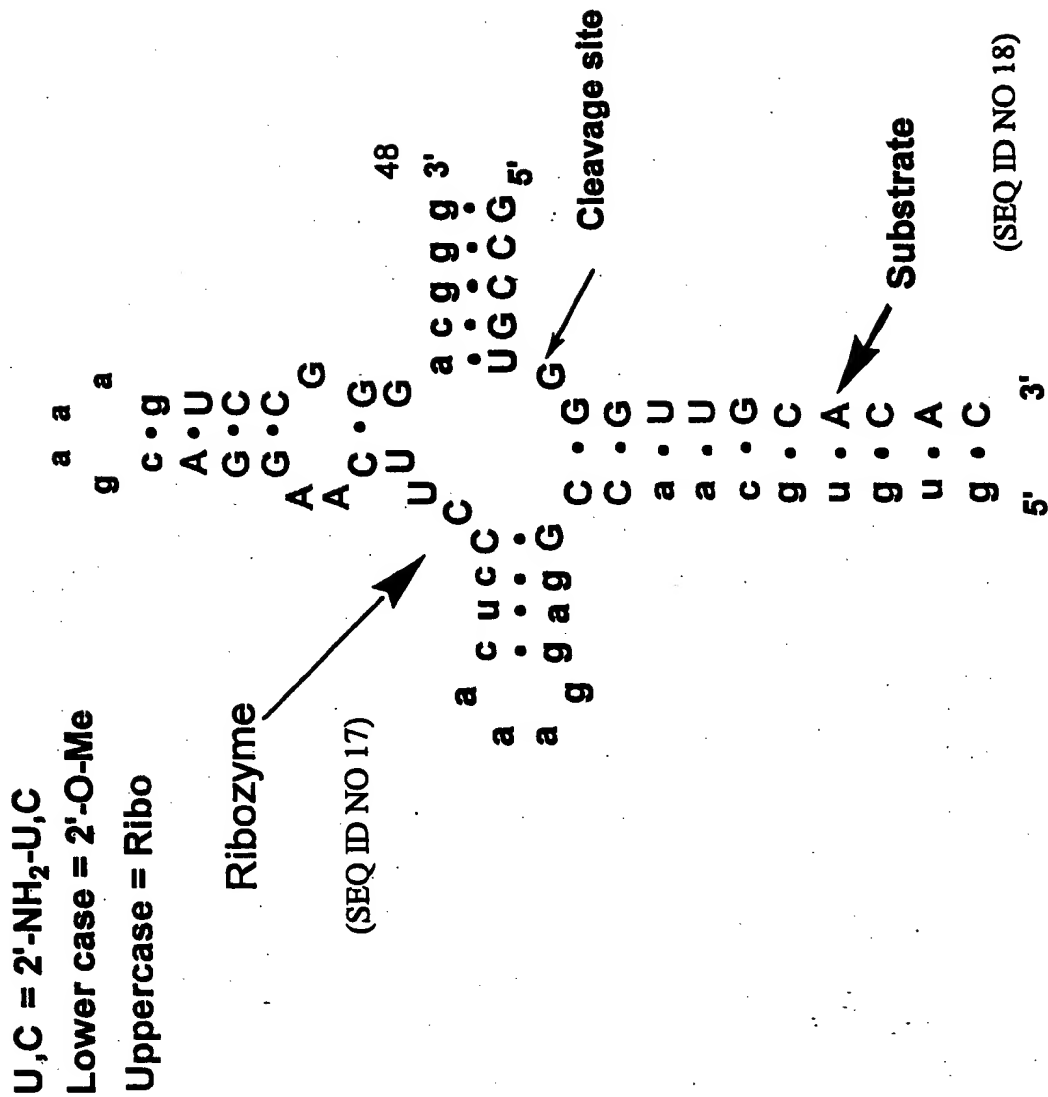
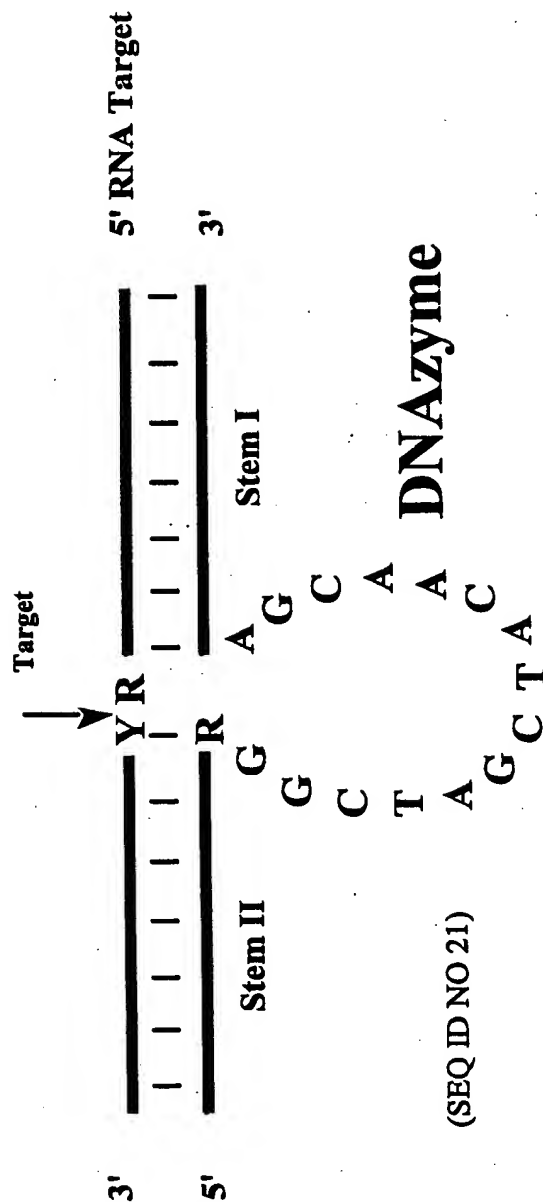


Figure 4: DNase Motif



Legend

Y = U or C
R = A or G

Figure 5. Detection of Target Sequence Using a Cis-Blocking Sequence

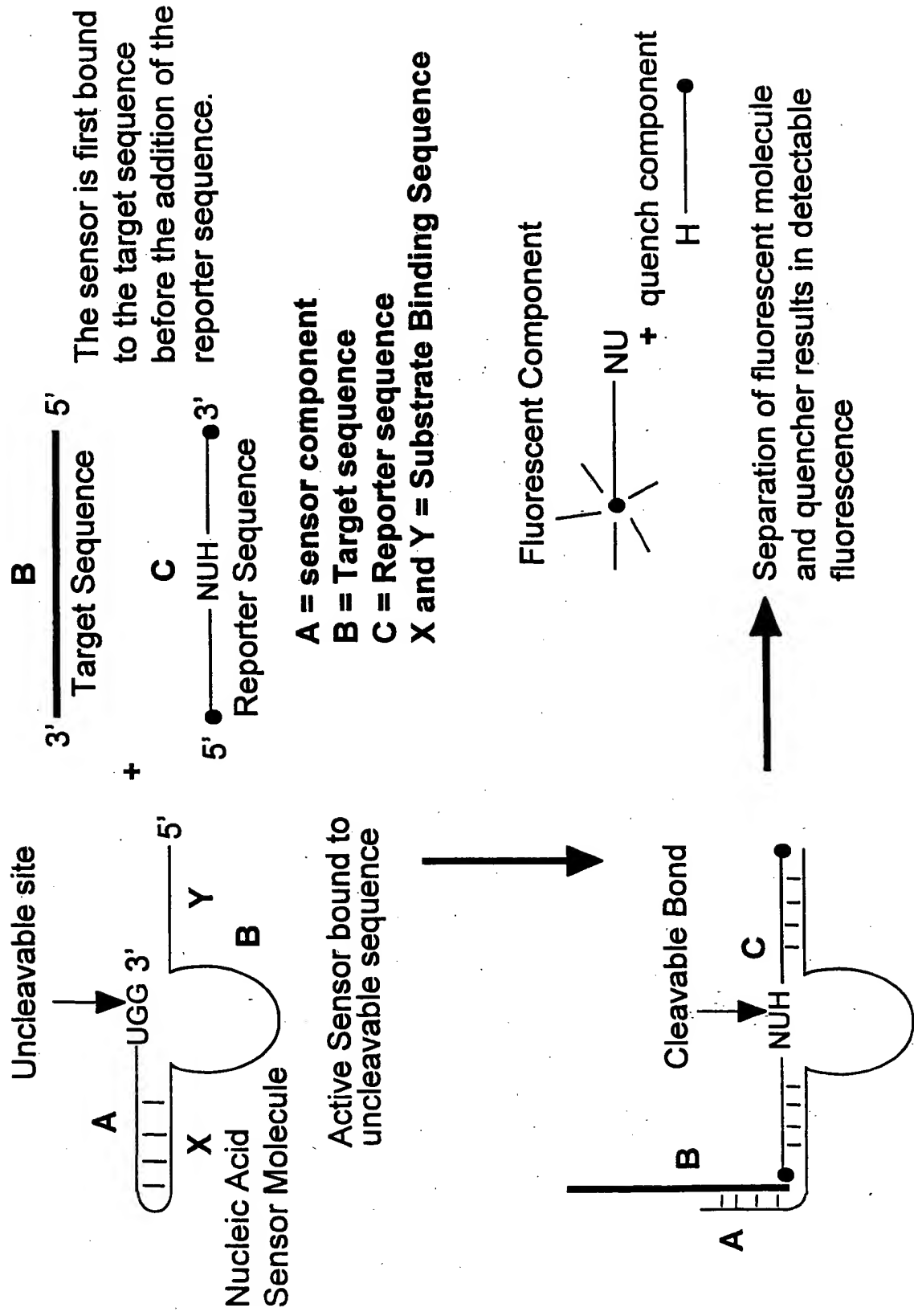


Figure 6. Schematic Diagram Representing the Two Primary Configurations of the Diagnostic effector molecule

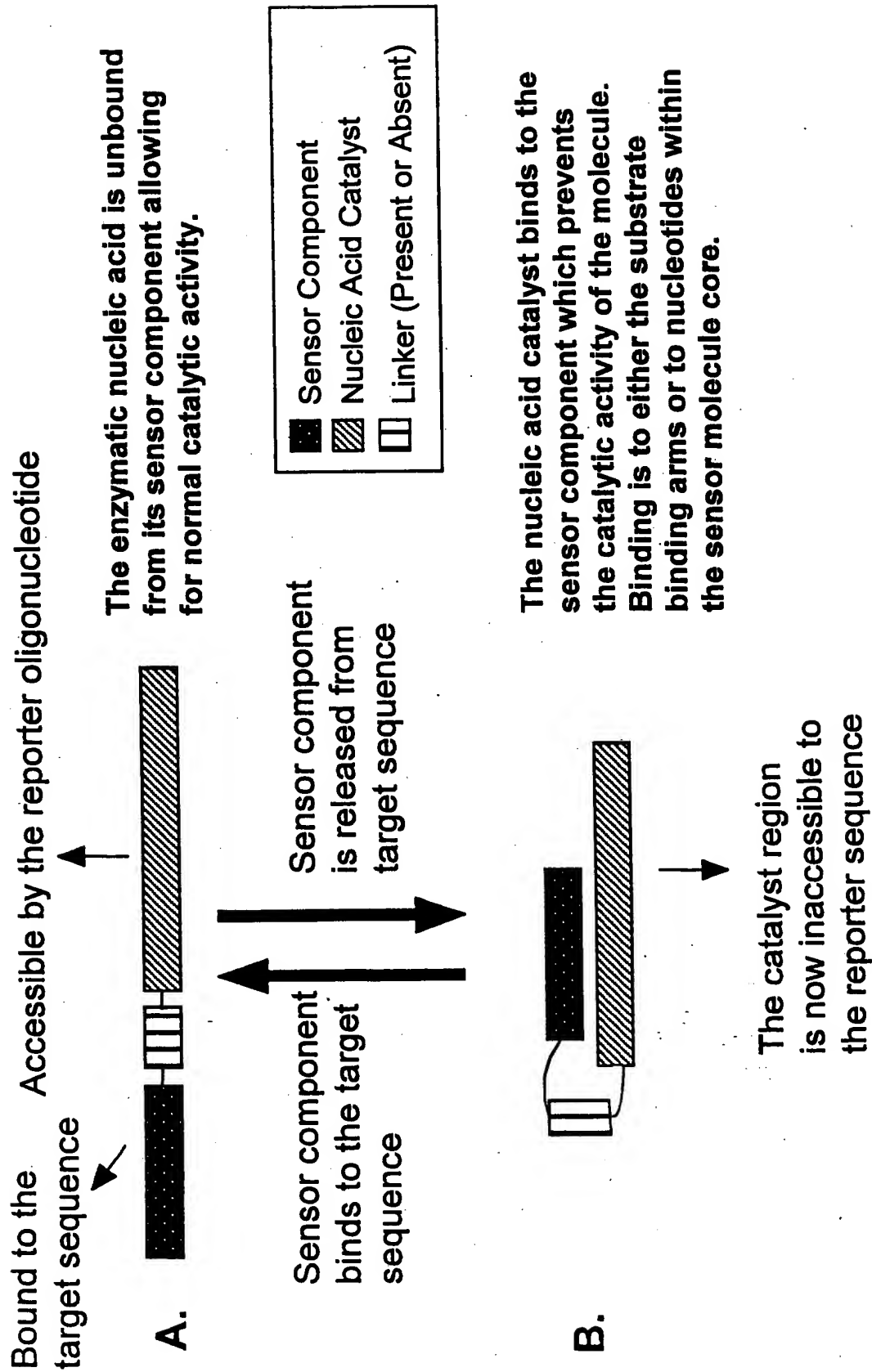


Figure 7. Examples of Nucleic Acid Sensor Molecules

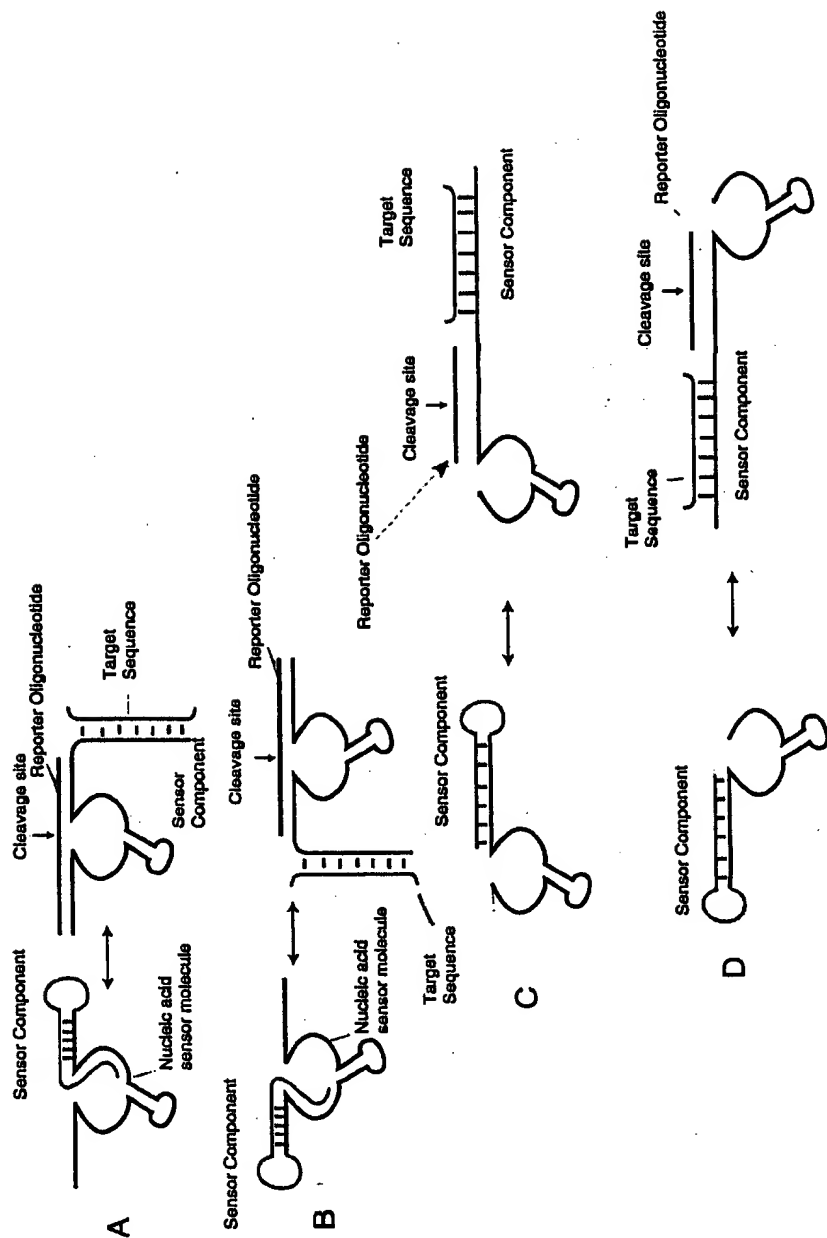


Figure 8. Examples of Nucleic Acid Sensor Molecules

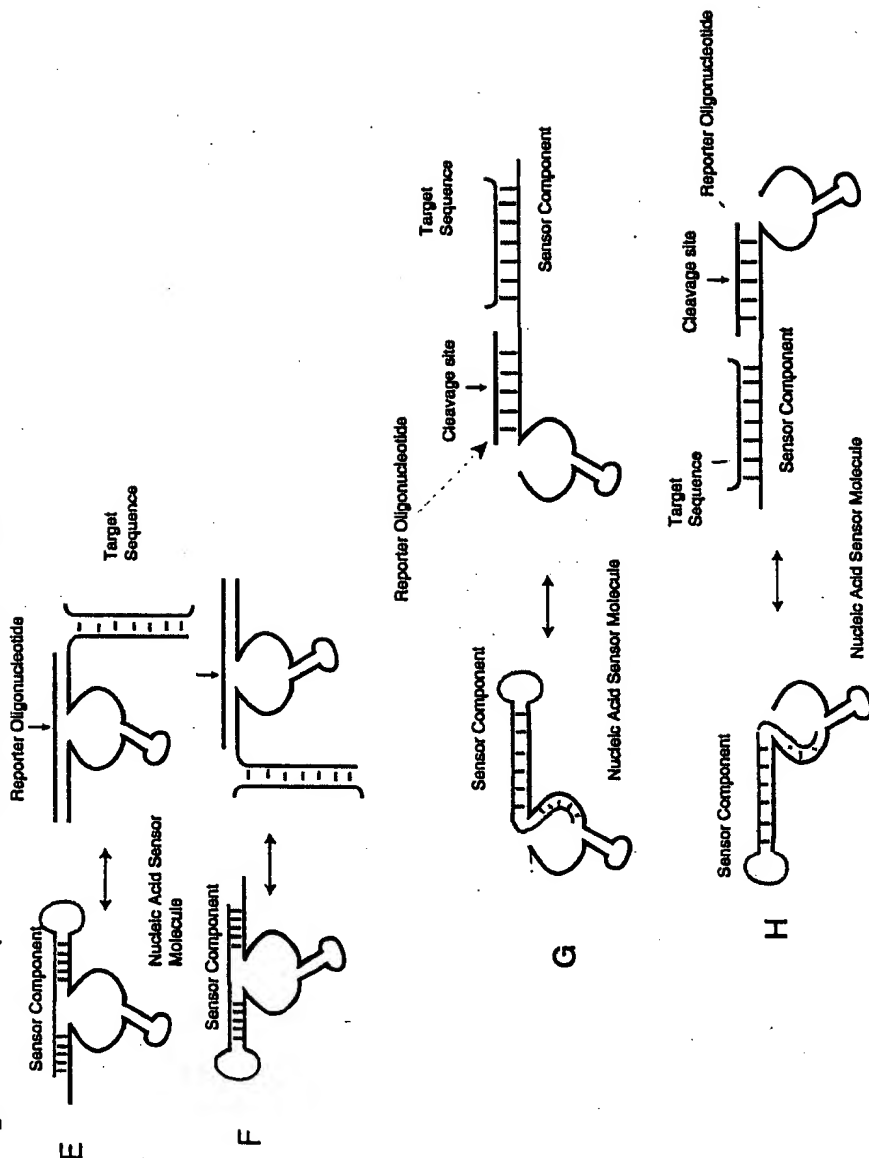


Figure 9. Examples of Nucleic Acid Sensor Molecules

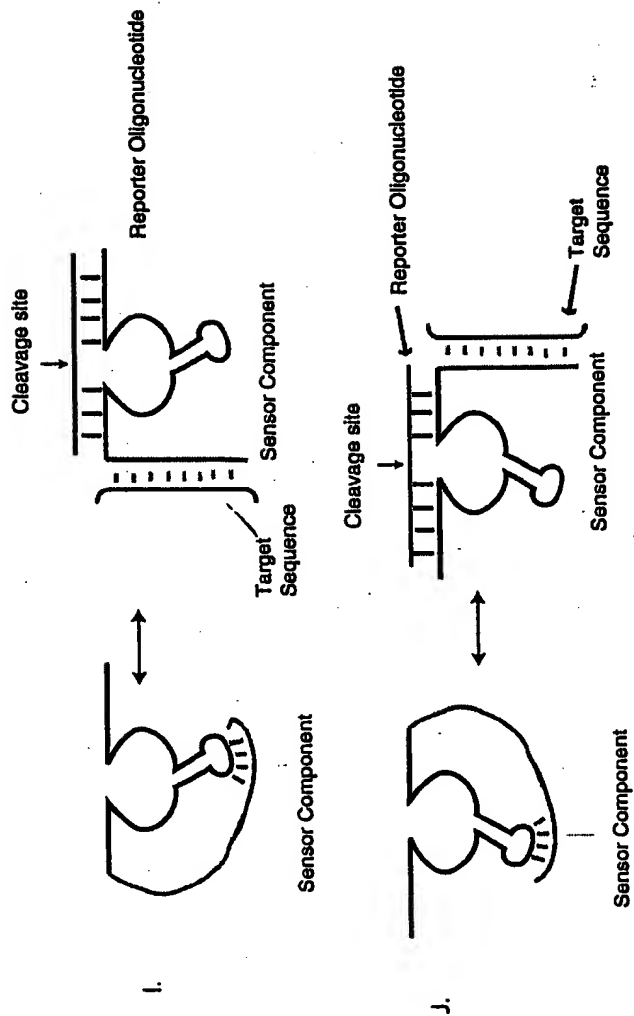


Figure 10: Examples of Nucleic Acid Sensor Molecules

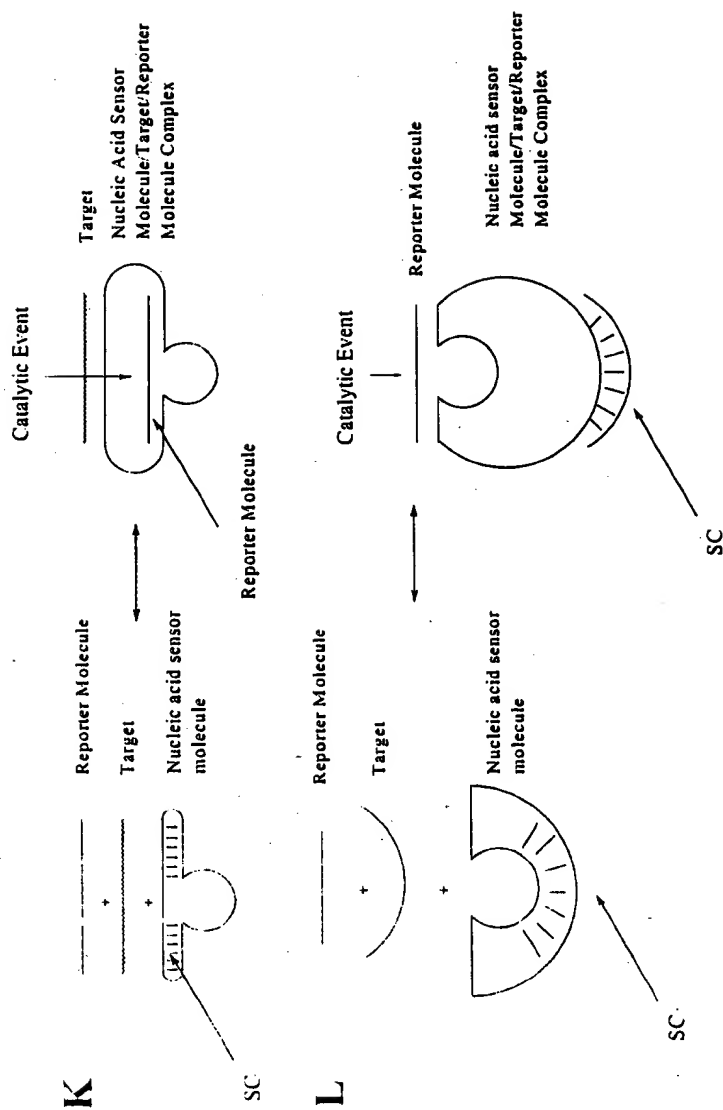


Figure 11: Examples of Nucleic Acid Sensor Molecules

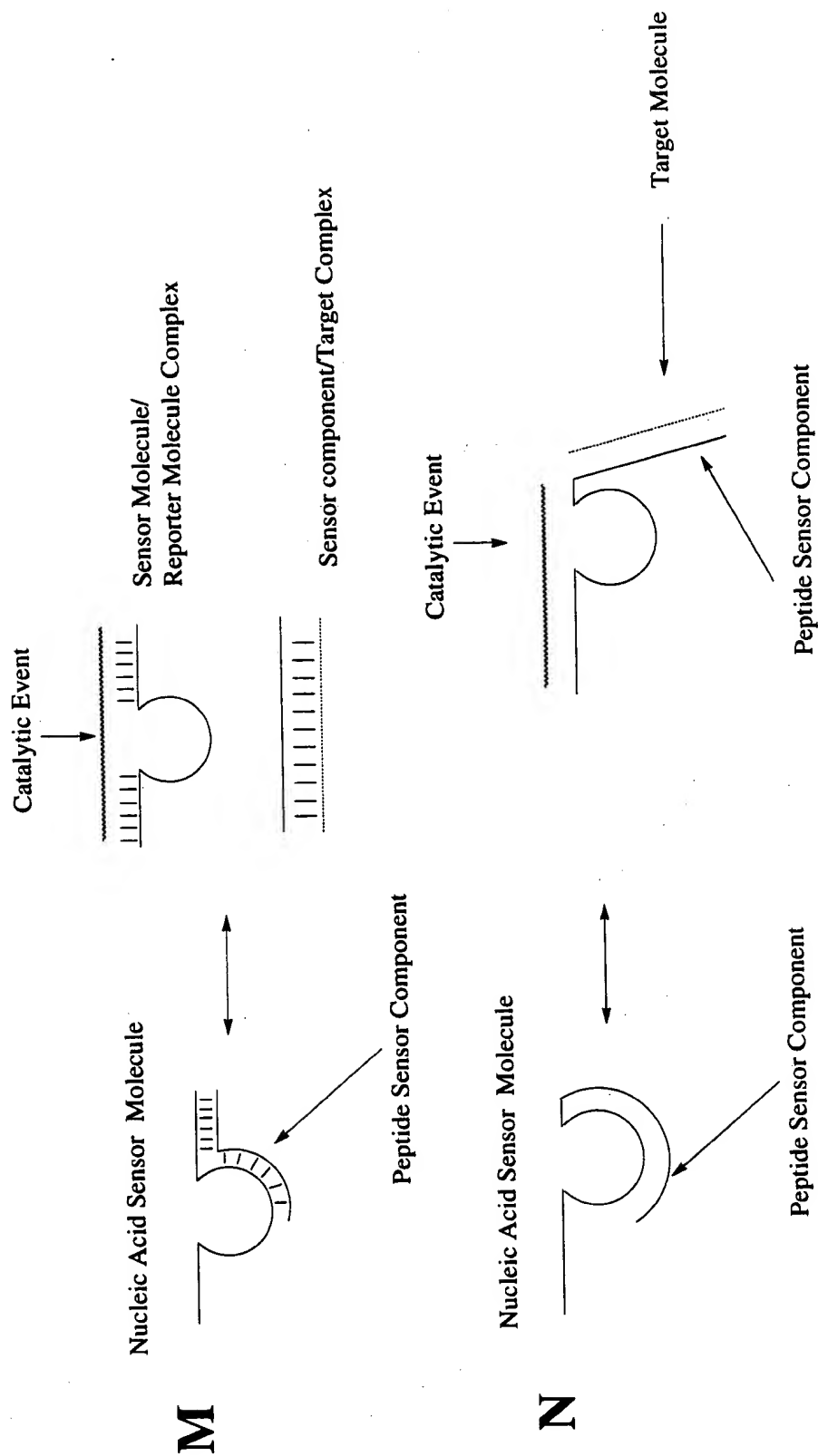
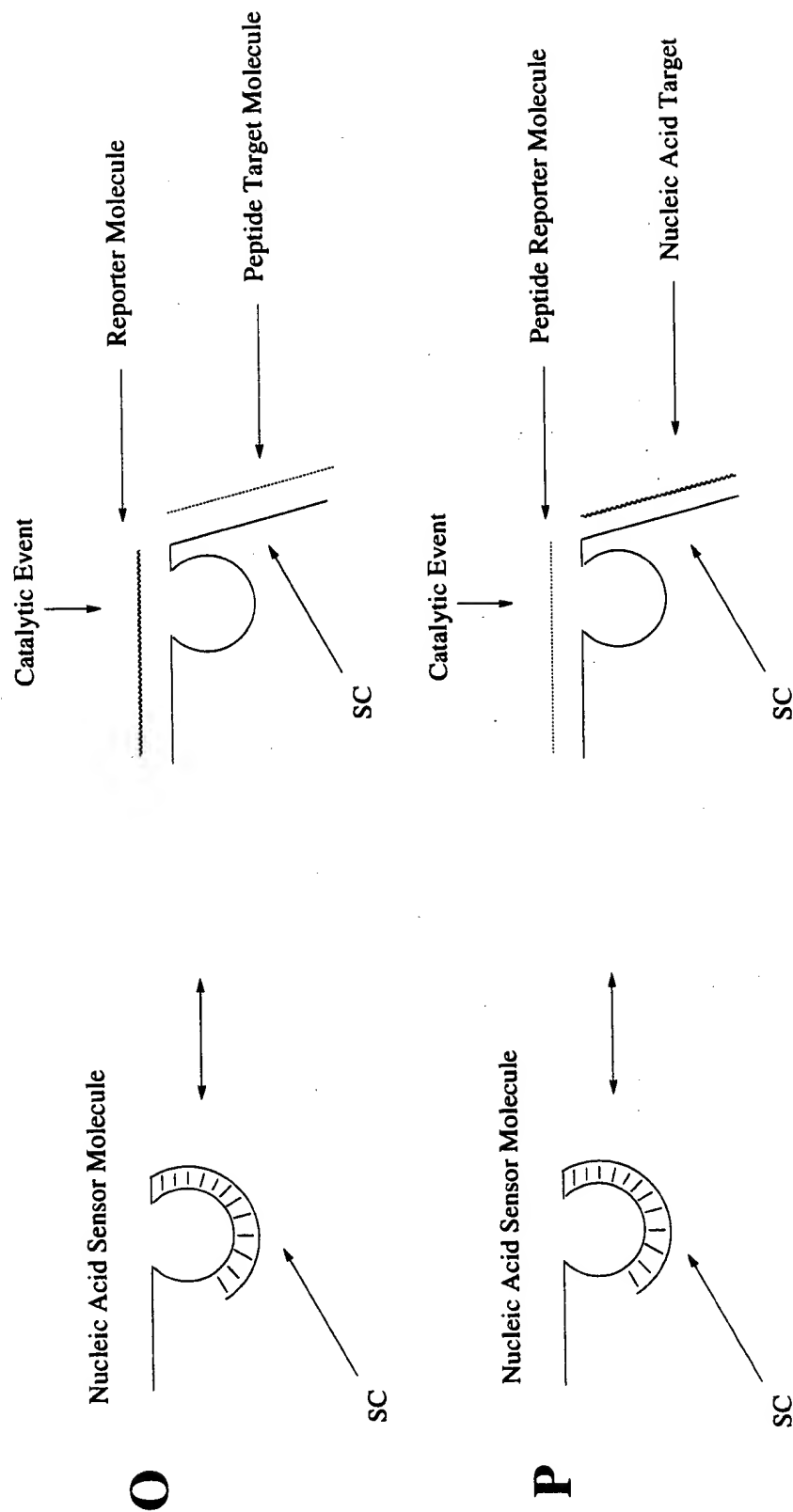


Figure 12: Examples of Nucleic Acid Sensor Molecules



SC = Sensor Component

Figure 13: Examples of Nucleic Acid Sensor Molecules

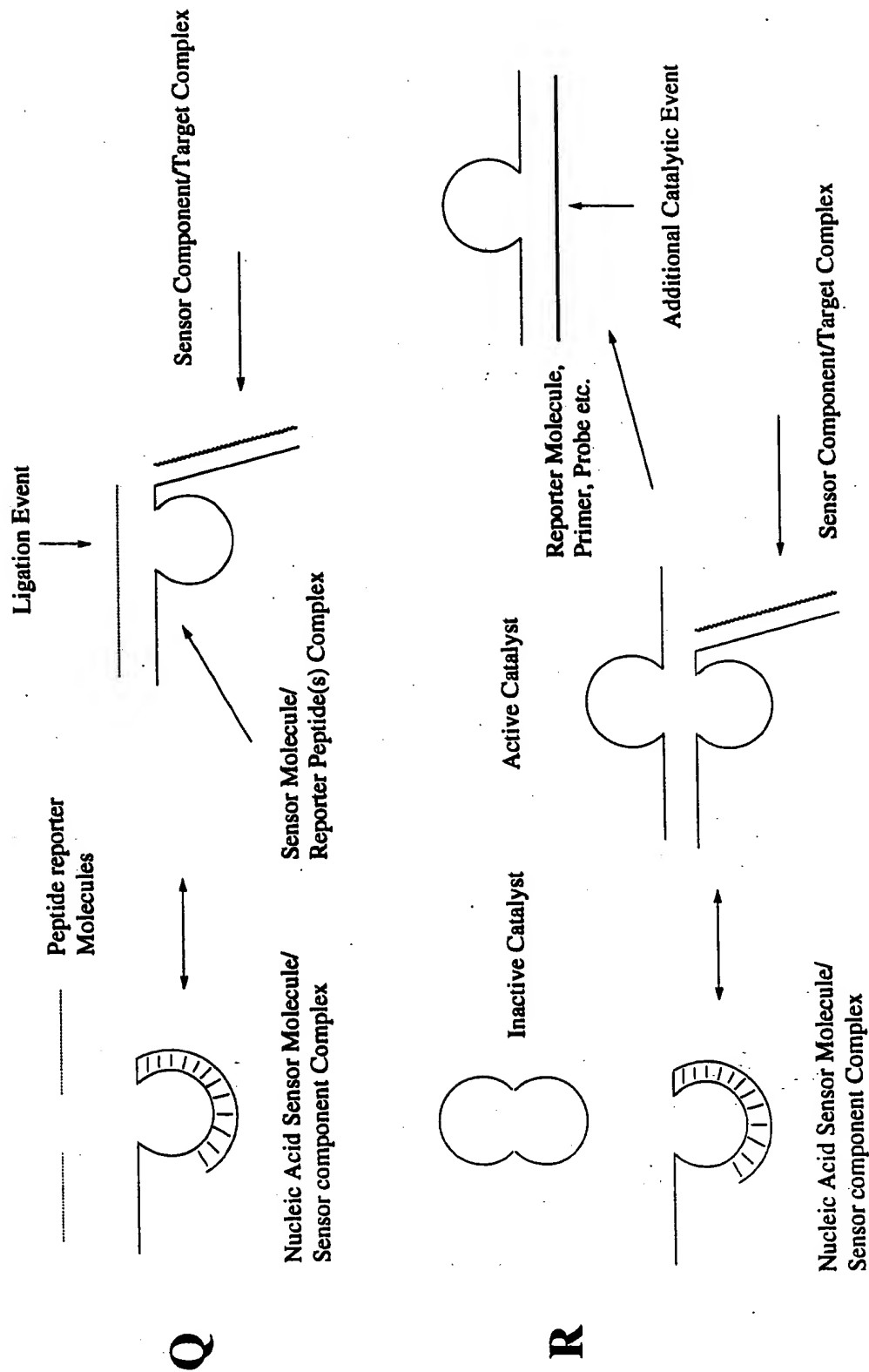


Figure 14: Inherent Amplification of Signal

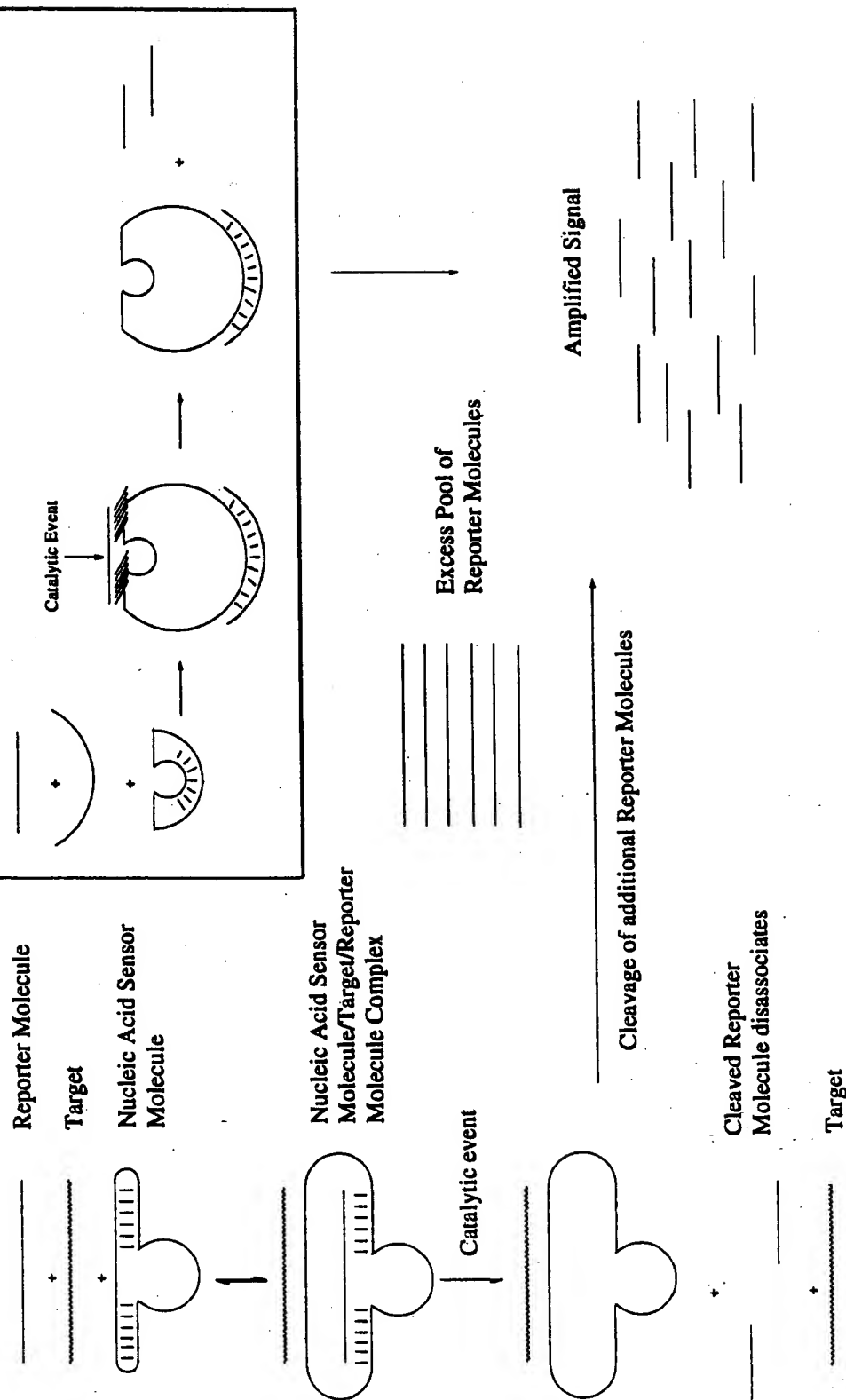


Figure 15: Example of Diagnostic System

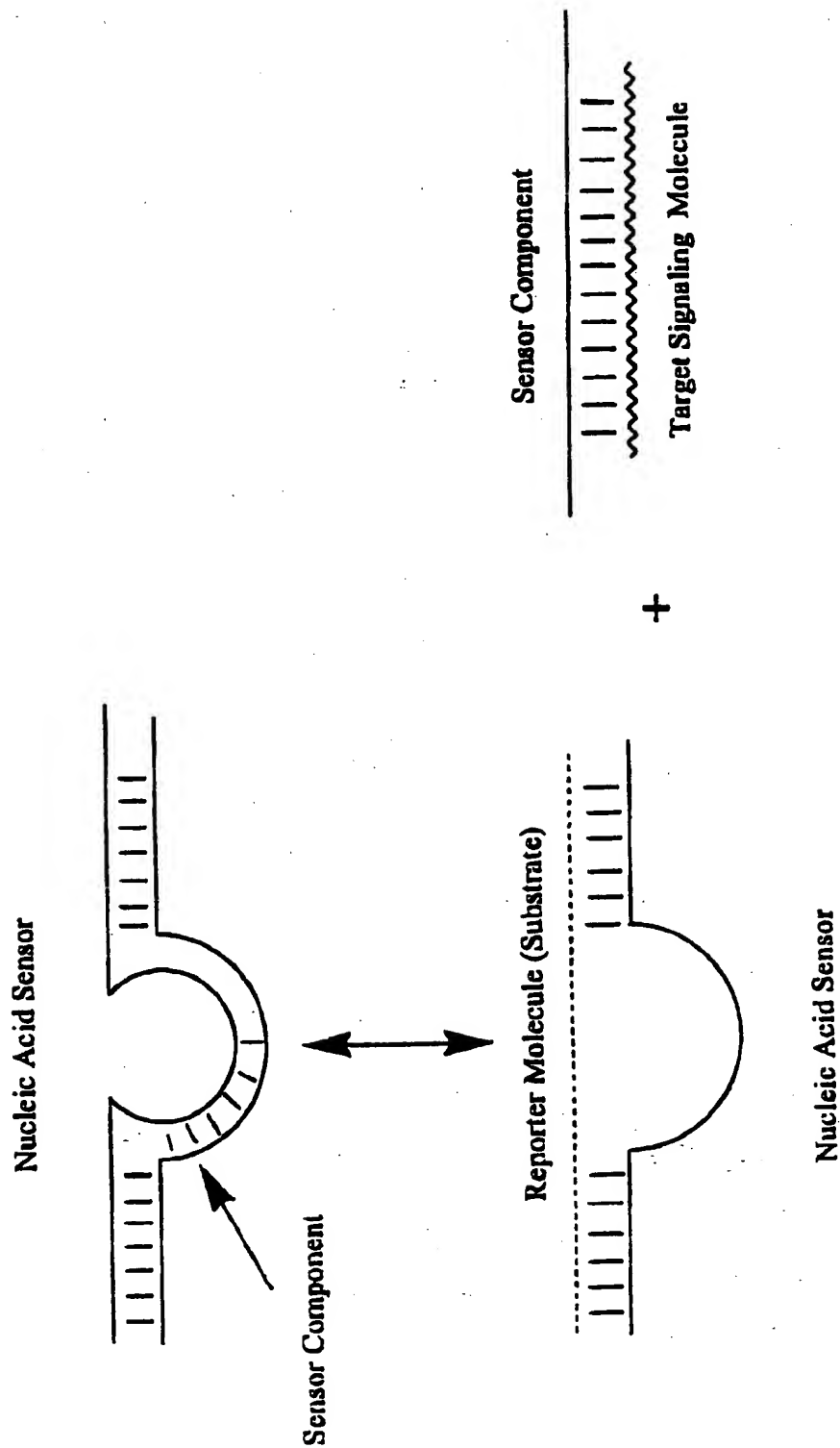
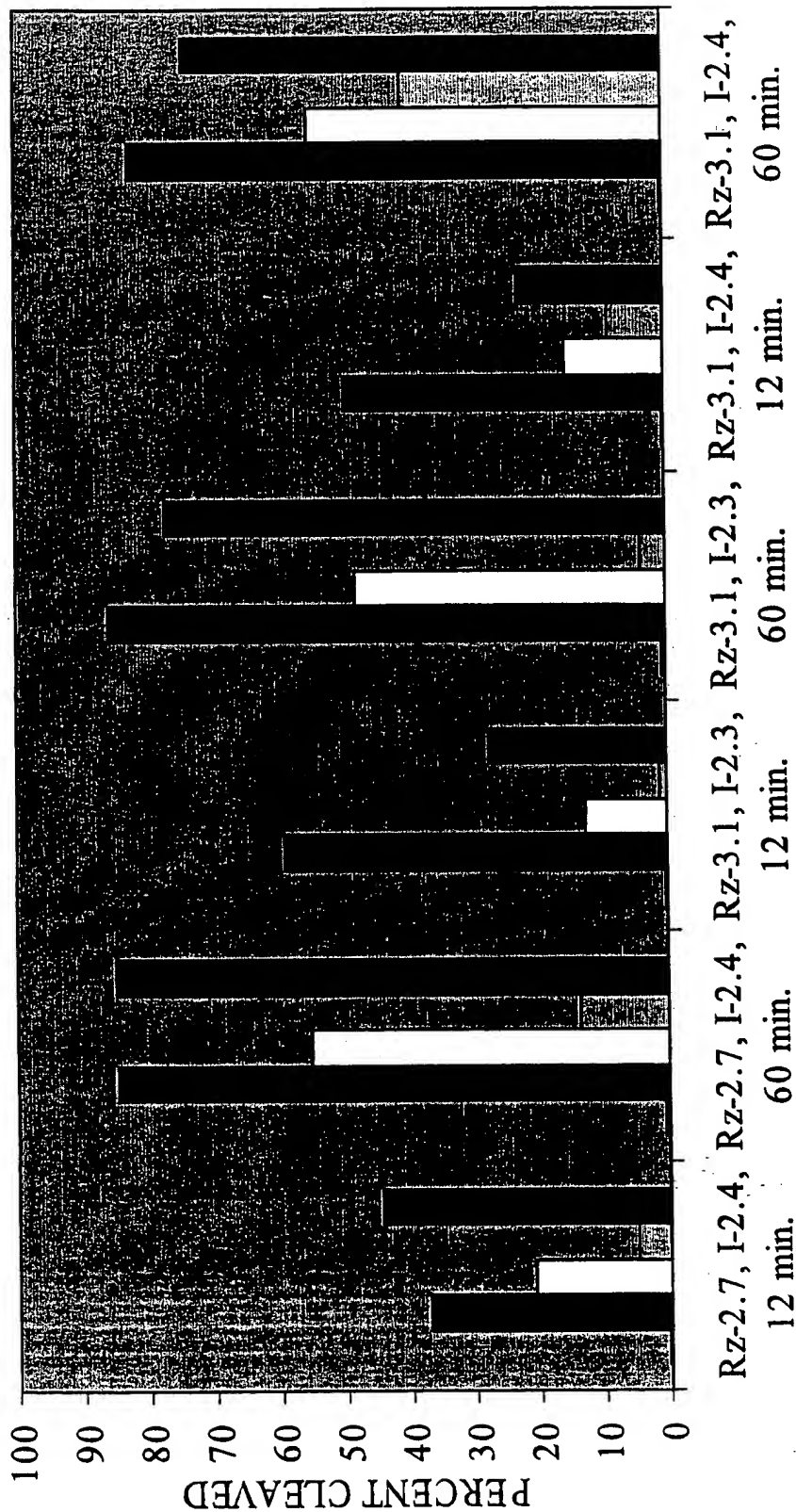


Figure 16: Ribozyme Diagnostic Screen

INHIBITORY FOLDING WITH TARGET RESCUE



No Rz
 +Rz @ 10 nM
 +Rz, +I @ 200 nM
 +Rz, +I, +T @ 500 nM

Figure 17a: Auto-ligation Nucleic Acid Sensor Molecules - Selection Scheme

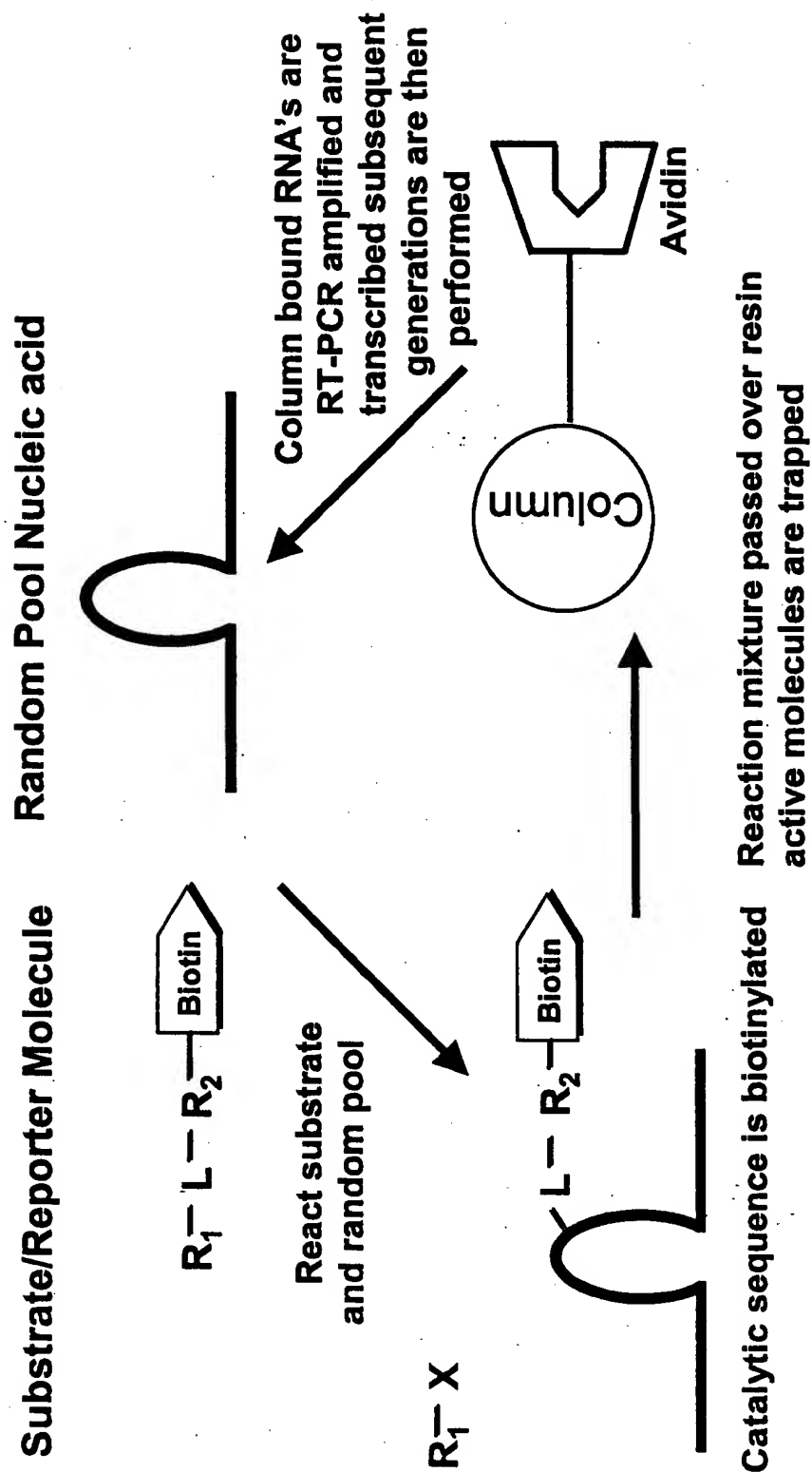
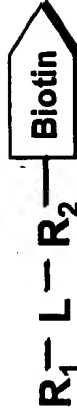


Figure 17b: Auto-ligation Nucleic Acid Sensor Molecules - Ligand Dependent

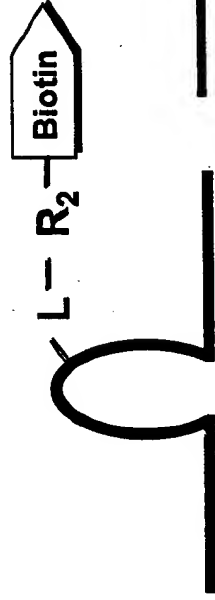
Substrate/Reporter Molecule + Random Pool Nucleic acid

- Ligand (first round)
- + Ligand (second round)



React substrate
and random pool

$R_1 - X$



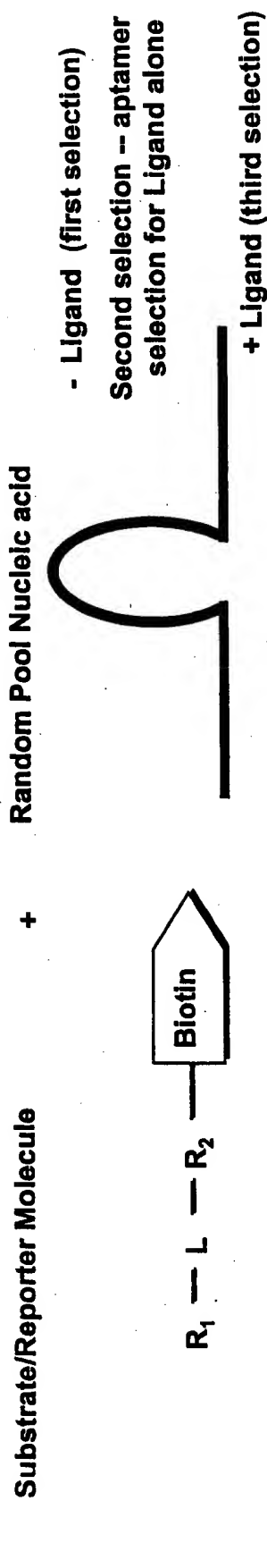
Catalytic sequence is biotinylated

Reaction mixture passed over resin
active molecules are trapped

Scheme I

Perform this reaction (in the absence of the Ligand) and disregard the molecules that bind to the avidin resin.
Collect all RNA's that flow through the avidin resin and repeat the reaction in the presence of the Ligand. Collect and RT-PCR amplify and transcribe these molecules for subsequent rounds.

Figure 17c: Auto-ligation Nucleic Acid Sensor Molecules - Ligand dependent



Scheme II

- Perform an entire selection as shown in first slide (in the absence of the Ligand)
- Mutagenize the winning pool
- Perform an entire selection using this pool with the requirement of Ligand binding.
- Mutagenize this pool
- Repeat original selection (for activity) in the presence of Ligand - countselect for molecules that react in the absence of ligand

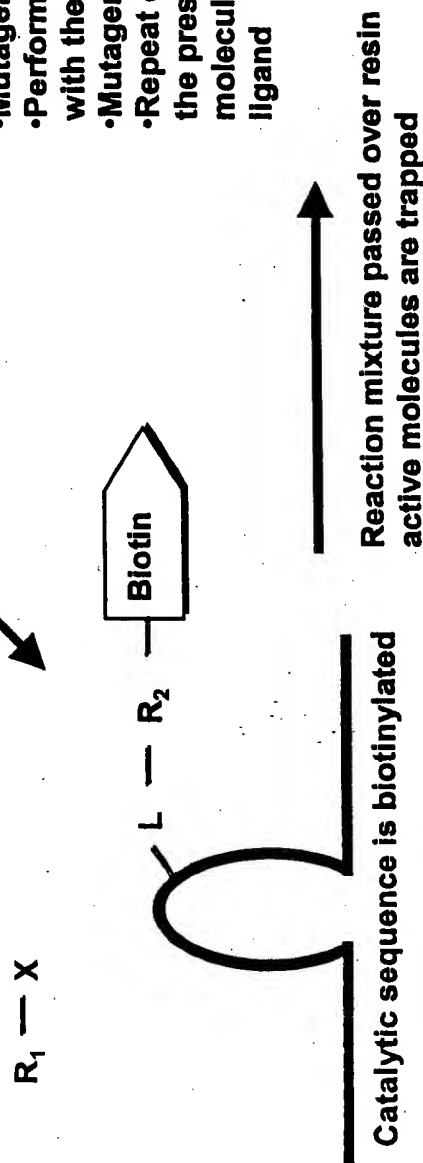


Figure 18a: Isomerase Nucleic Acid Sensor Molecule – Selection Scheme

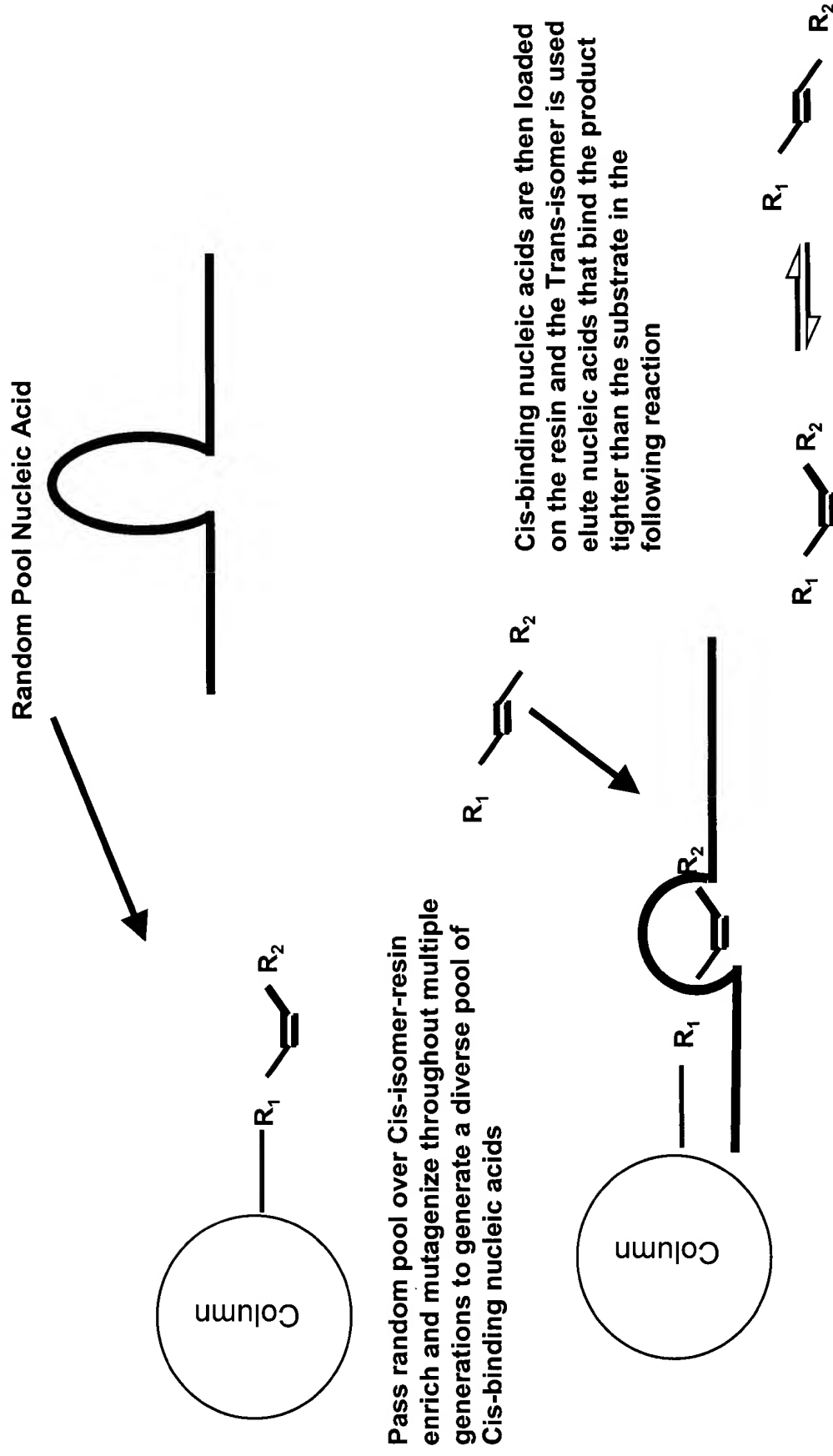
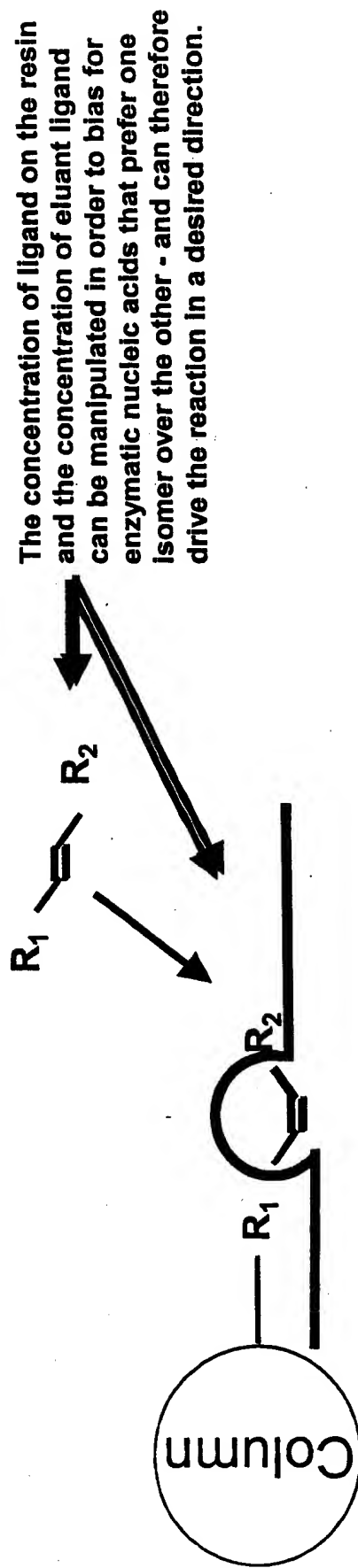


Figure 18b: Isomerase Nucleic Acid Sensor Molecule - Selection Scheme

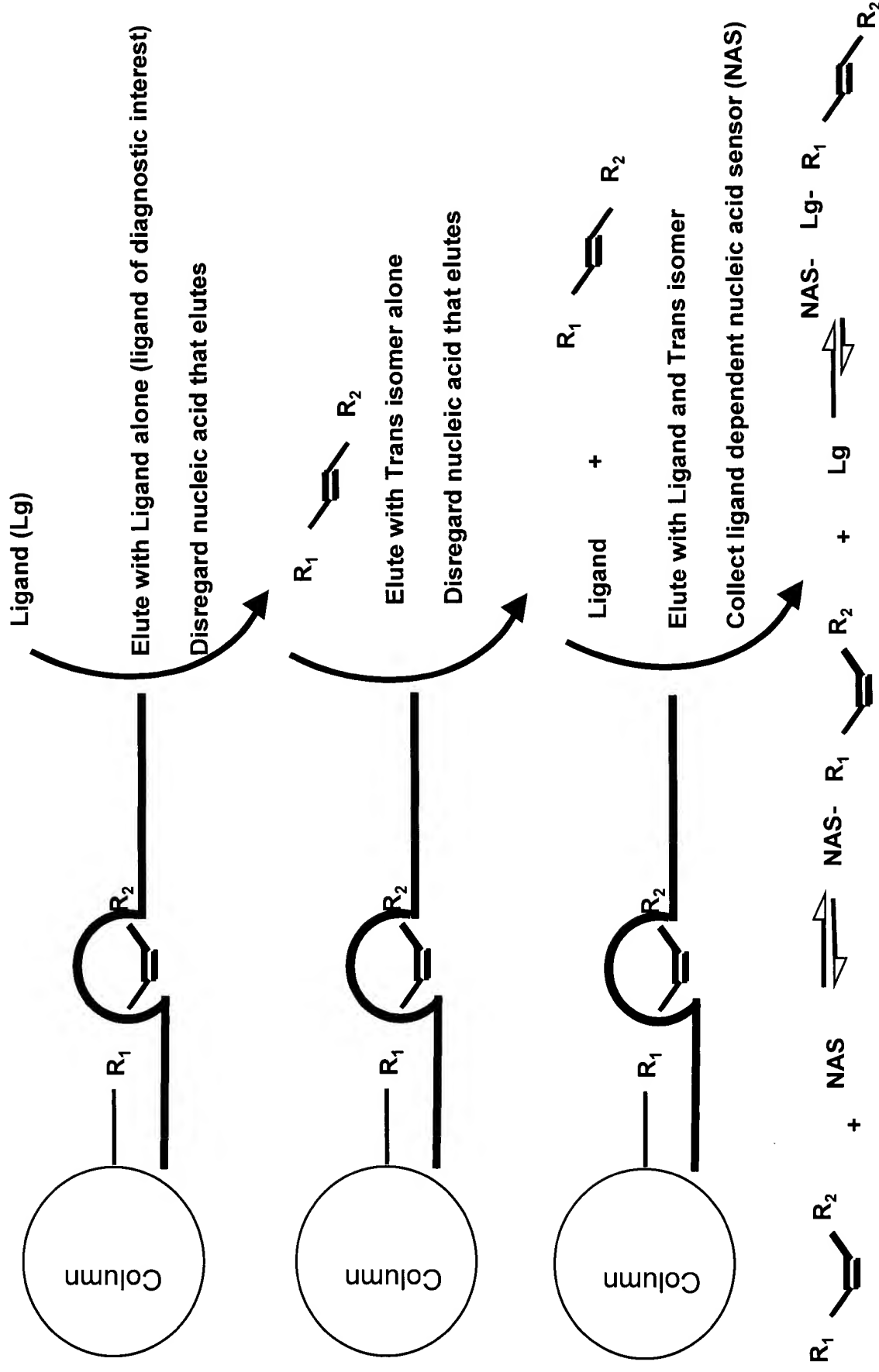


E.g. Selection for C_{is}-Isomer at 100 μ M - yield $K_d^{\text{cis}} = 100 \mu\text{M}$
 Elute with Trans-isomer at 0.1 μ M - yield $K_d^{\text{trans}} = 0.1 \mu\text{M}$

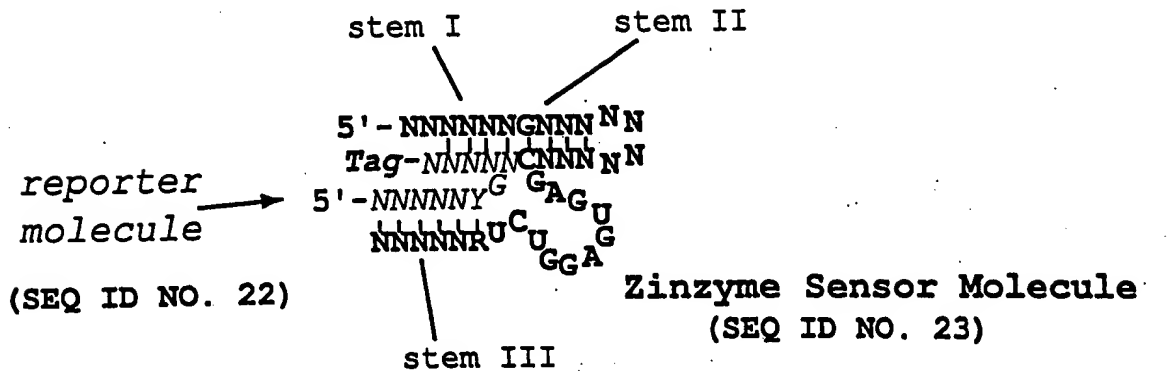
Isolate catalysts for the reaction below



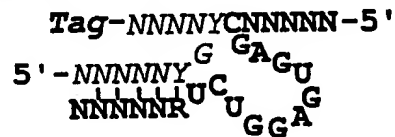
Figure 18c: Isomerase Nucleic Acid Sensor Molecule - Ligand dependent



Zinzyme Sensor Molecule for detection of Nucleic Acid



Inactive Zinzyme sensor/
reporter molecule complex
(SEQ ID NO. 22)



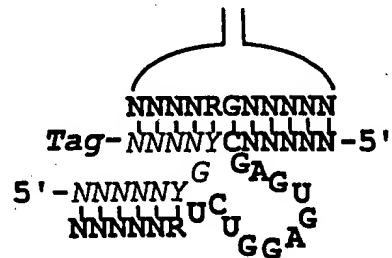
(SEQ ID NO. 24)

Target Signaling
Molecule



Target Signaling
Molecule

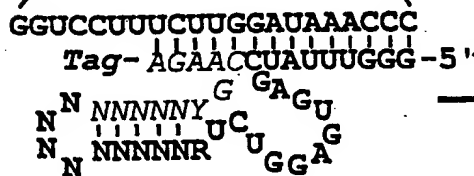
(SEQ ID NO. 25)



Active Zinzyme sensor/
reporter molecule complex

Stem-loop III of HCV (SEQ ID NO. 26)
(SEQ ID NO. 22)

Active HCV Zinzyme sensor/
reporter molecule complex
(SEQ ID NO. 27)

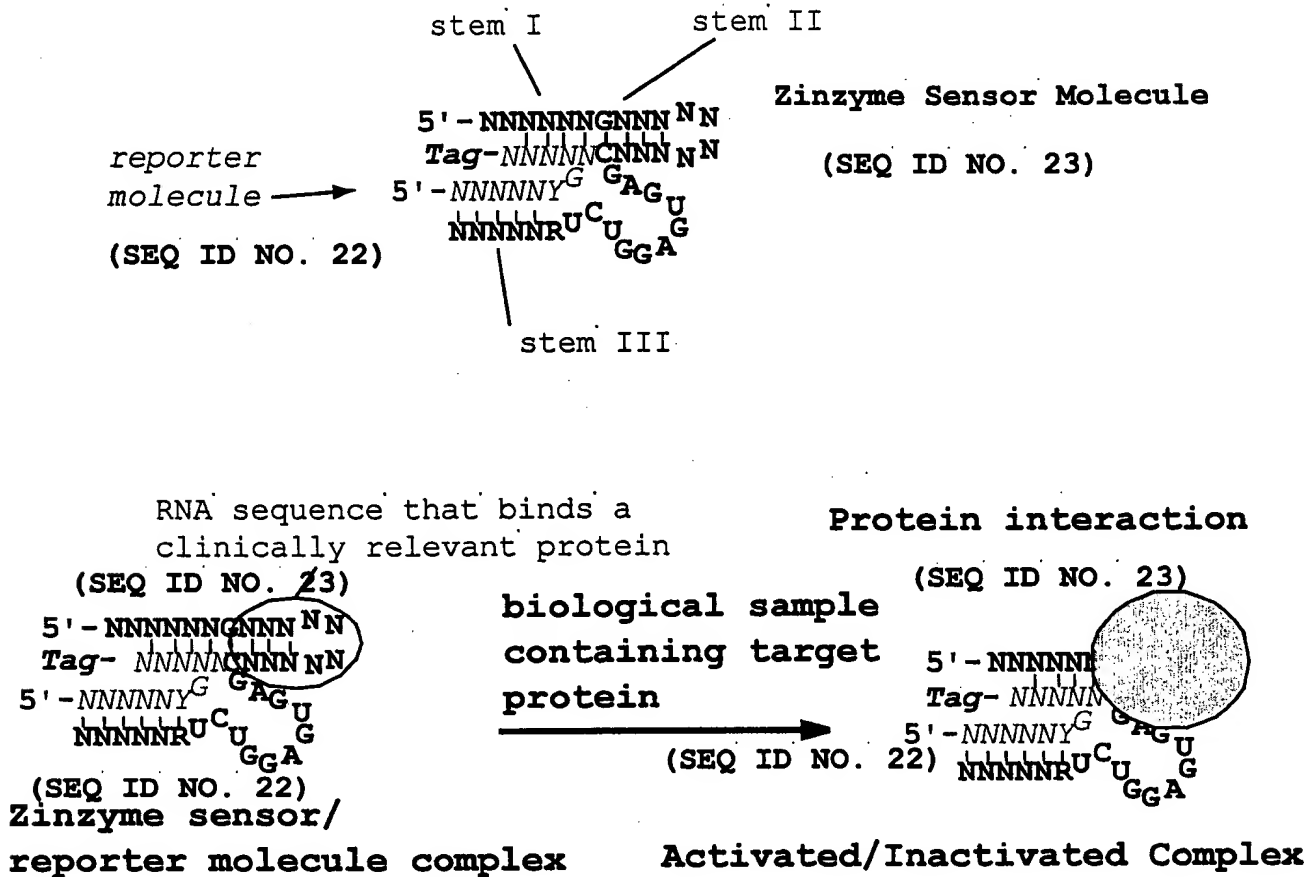


catalysis results in
release of Tag-AGAAC
for detection

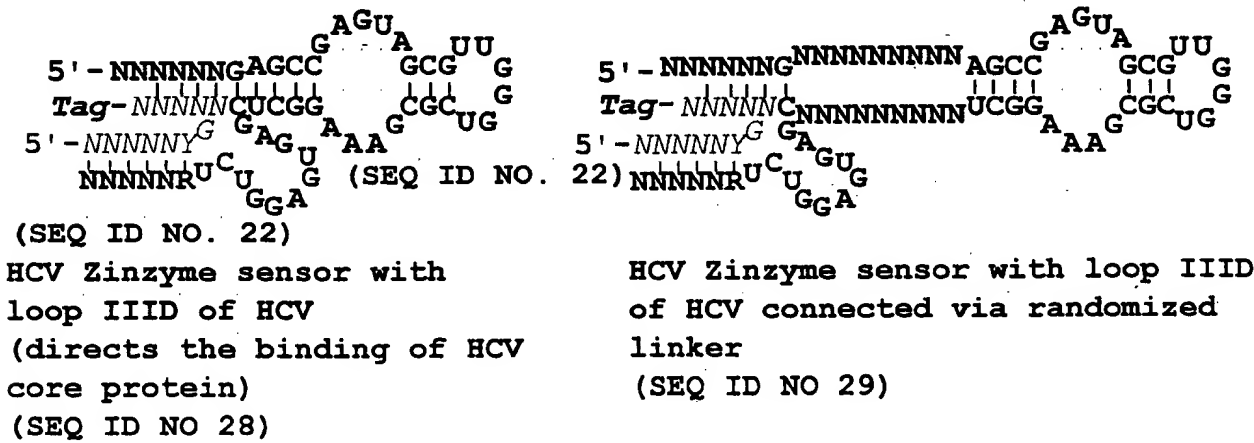
Zinzyme sensor can be attached to solid support/surface,
for example at the 5'-end.

FIG. 19

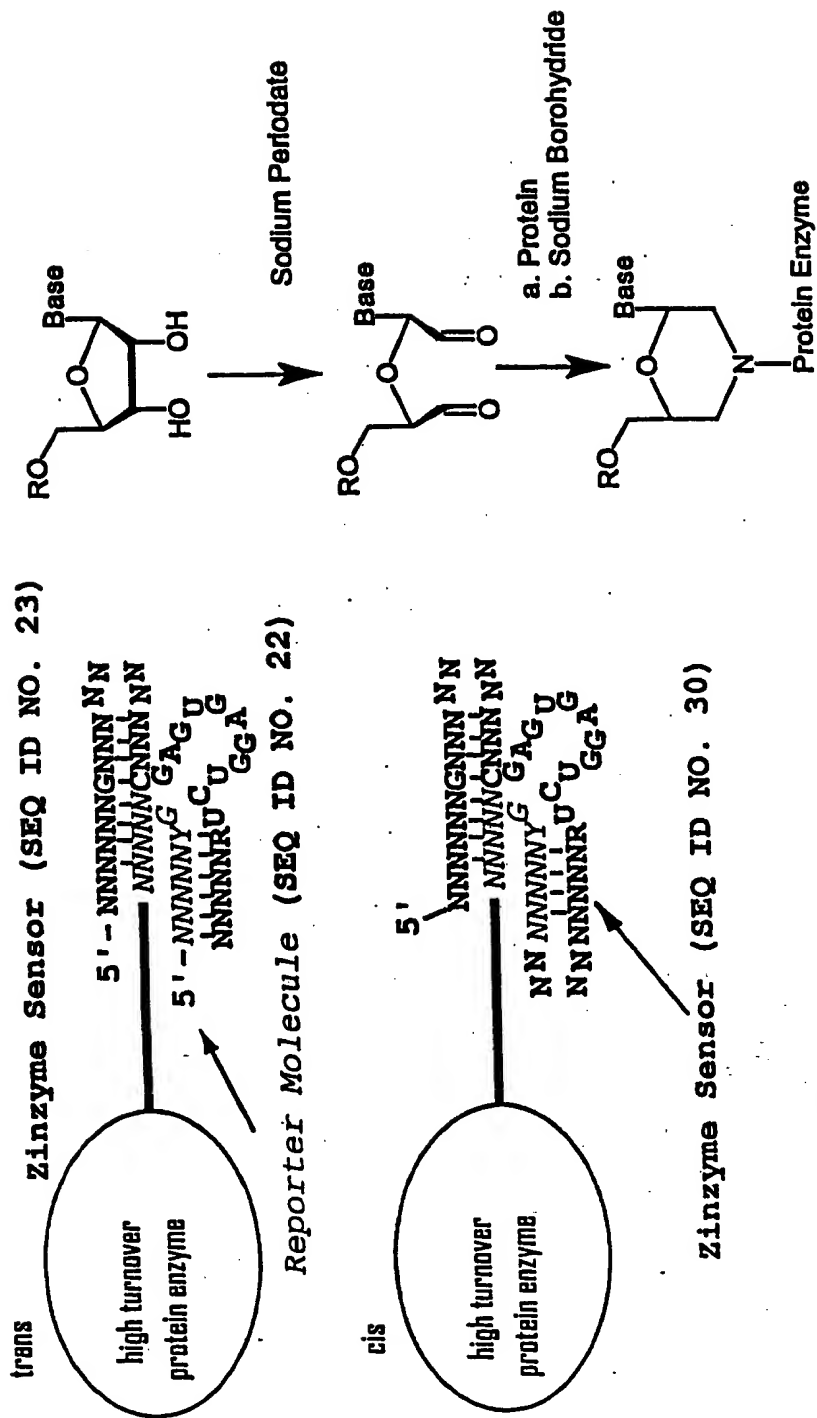
Figure 20: Zinzyme Sensor Molecule for detection of Protein



Sensor/reporter complex for detection of HCV core protein



Zinzyme Sensor Molecule with protein enzyme reporter



R is oligonucleotide.

Protein can be attached via amino linker.

Alternately, R is phosphoramidite moiety for incorporation at 5'-end of oligonucleotide.

High turnover protein enzyme is, for example, Luciferase, Horseshoe peroxidase, beta-galactosidase, alkaline phosphatase.

FIG. 21

Amplification of signal via use of protein enzyme conjugate

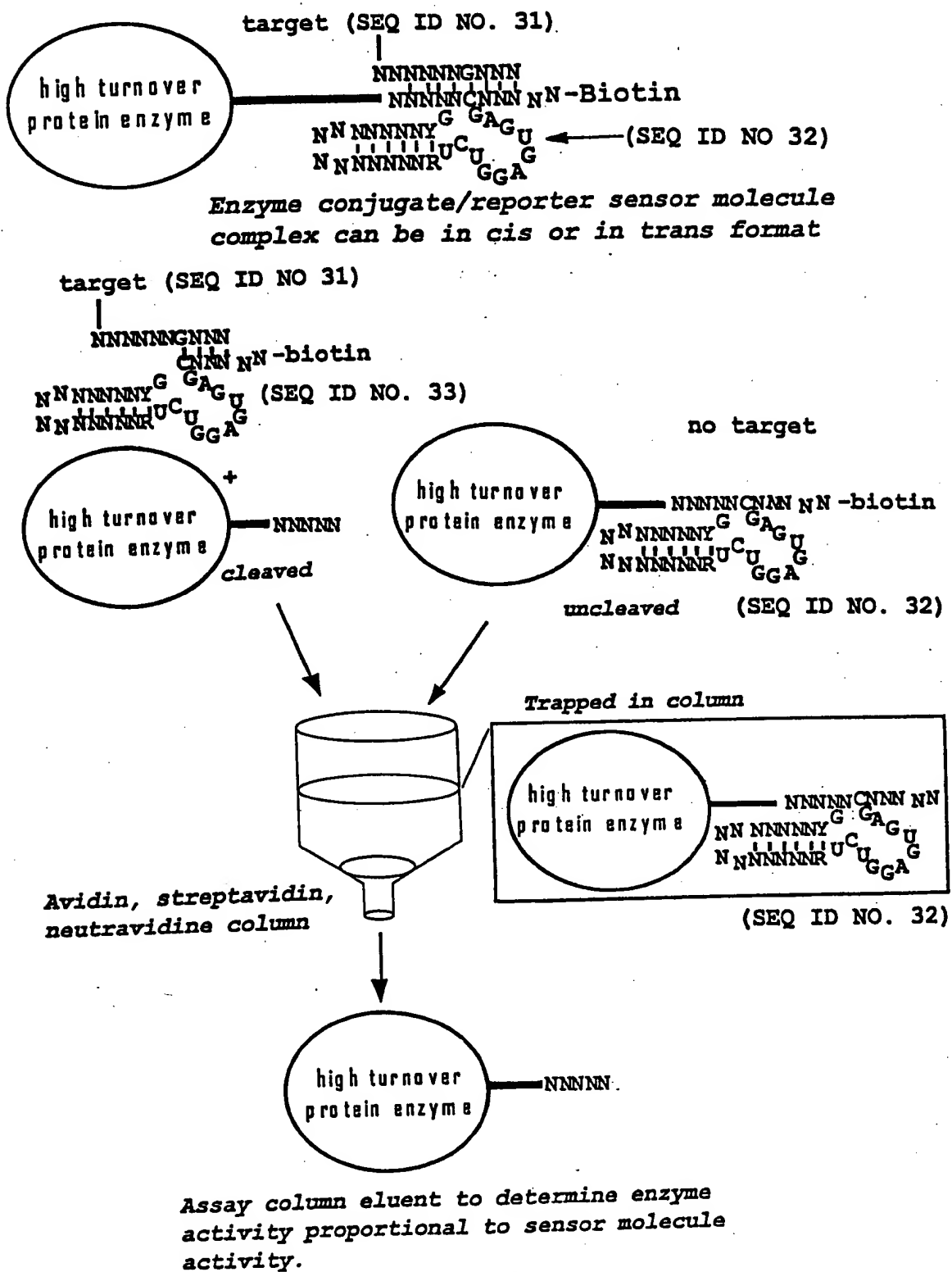
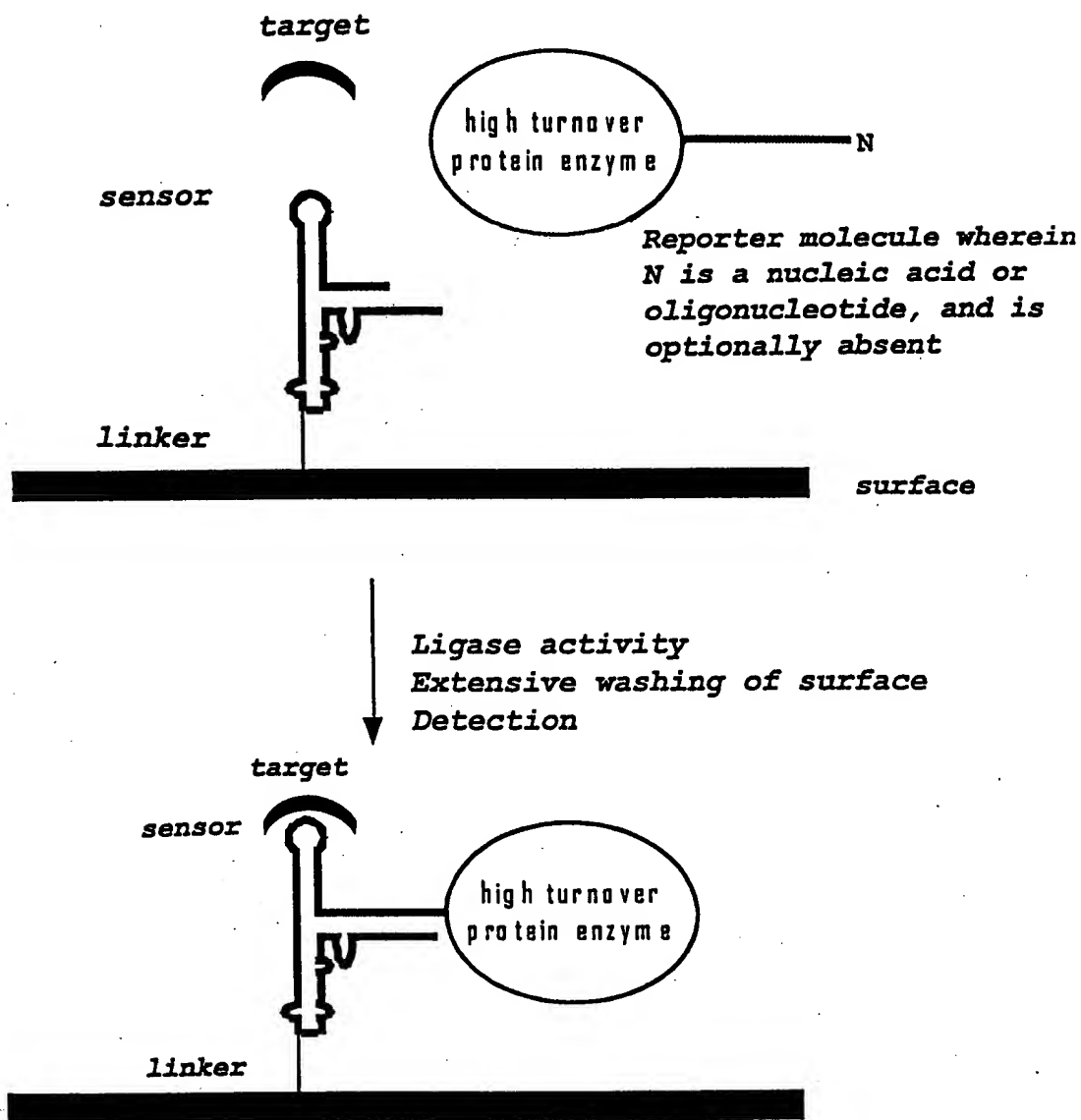


FIG. 22

0987536-100201

Ligase Sensor Molecule with enzymatic reporter



Alternatively, a fluorescent or chemiluminescent based reporter molecule is used.

FIG. 23

Figure 24: Selection of Nucleic Acid Sensor Molecules with Ligase Activity

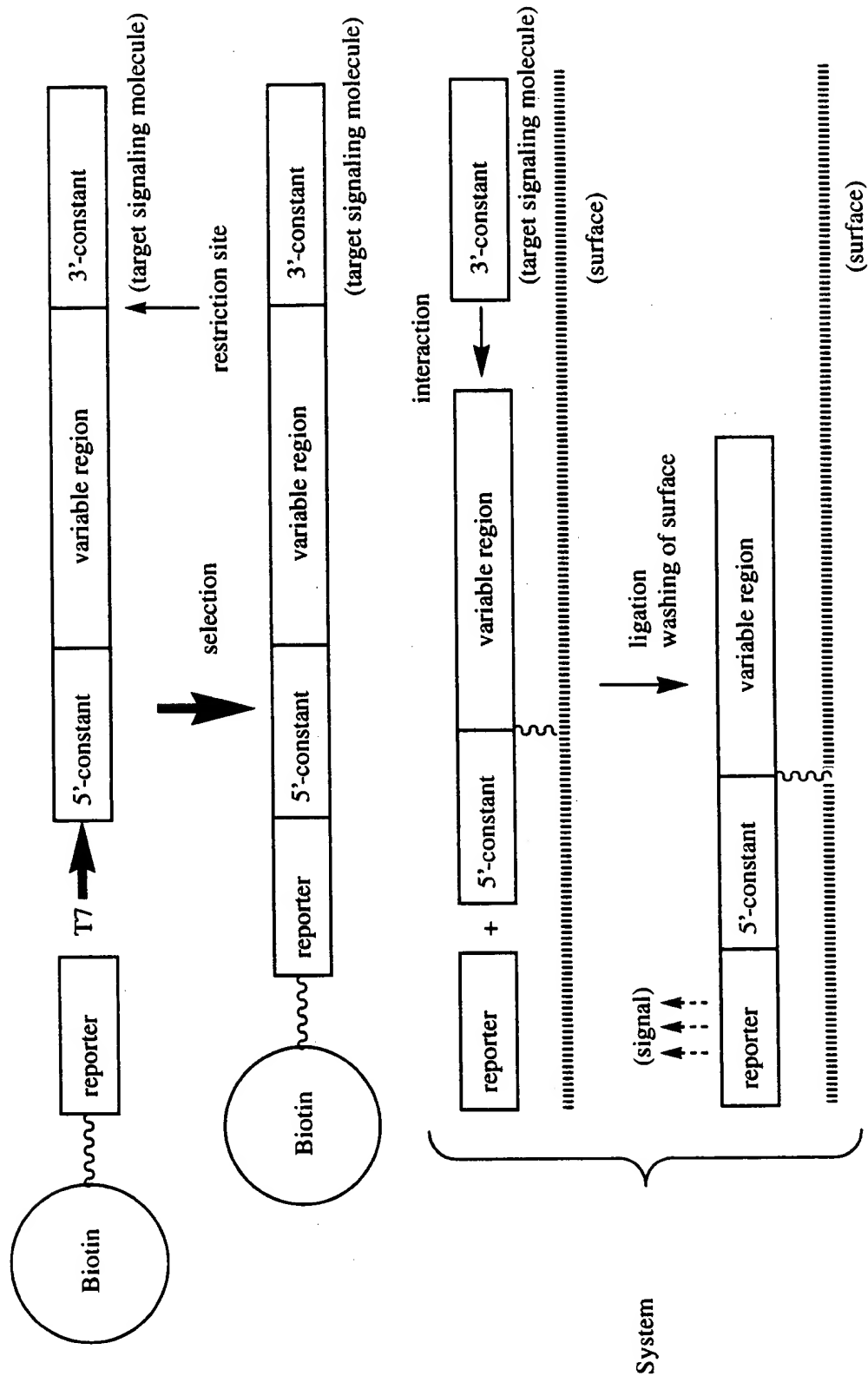
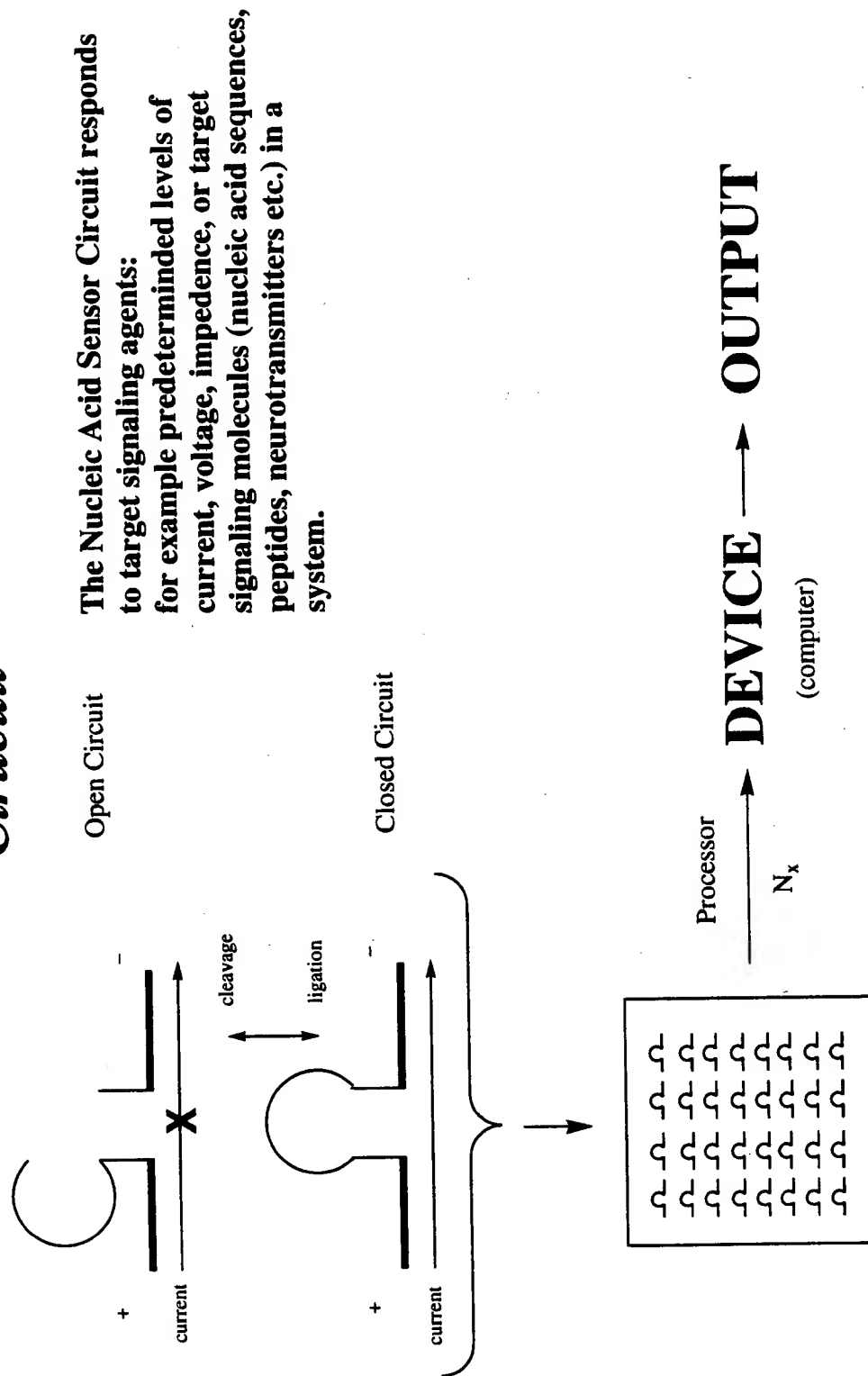


Figure 25: Nucleic Acid Sensor Molecule-Based Electric Circuit



ACTIVE ↔ TARGET INACTIVATED

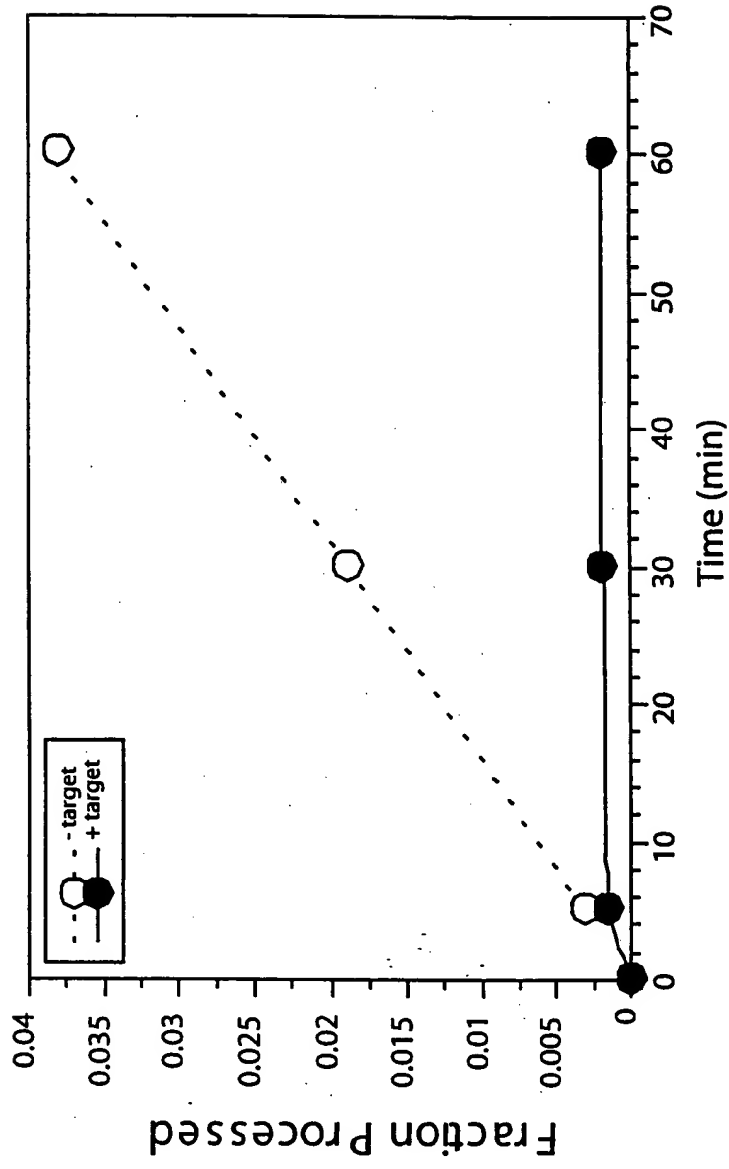


Figure 27: Target Activation of Zinzyme Sensor Molecule

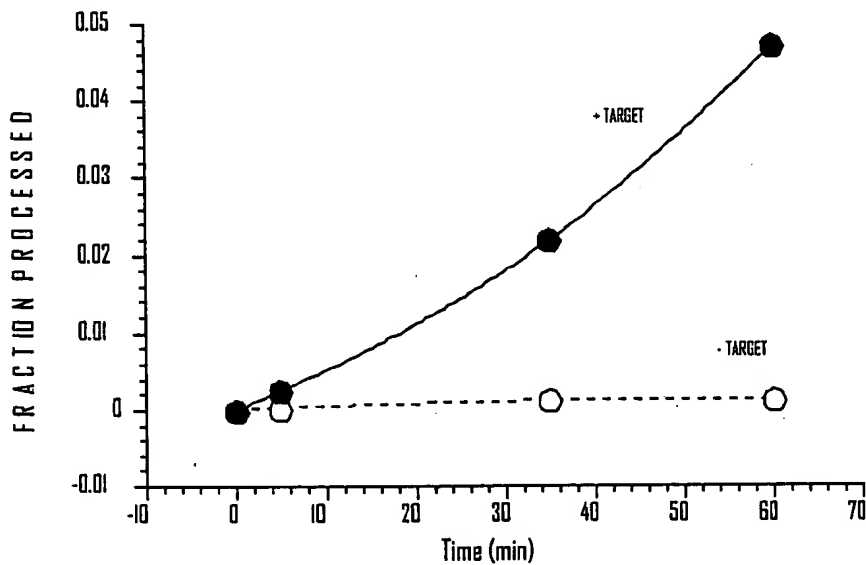
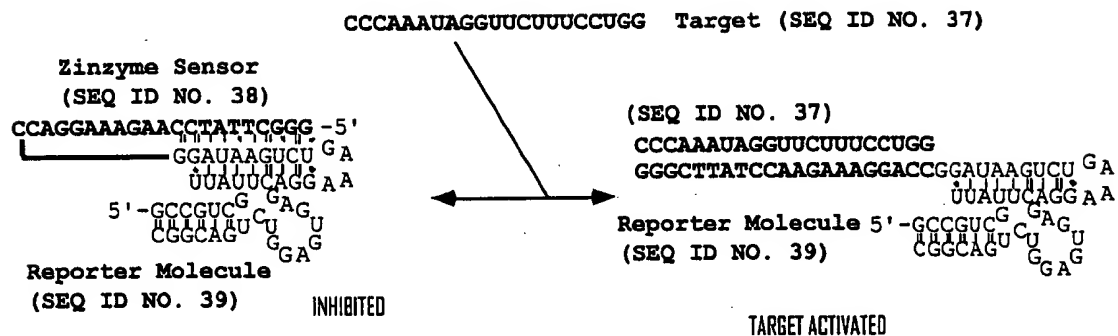


Figure 29: Half-Zinczyme Construct

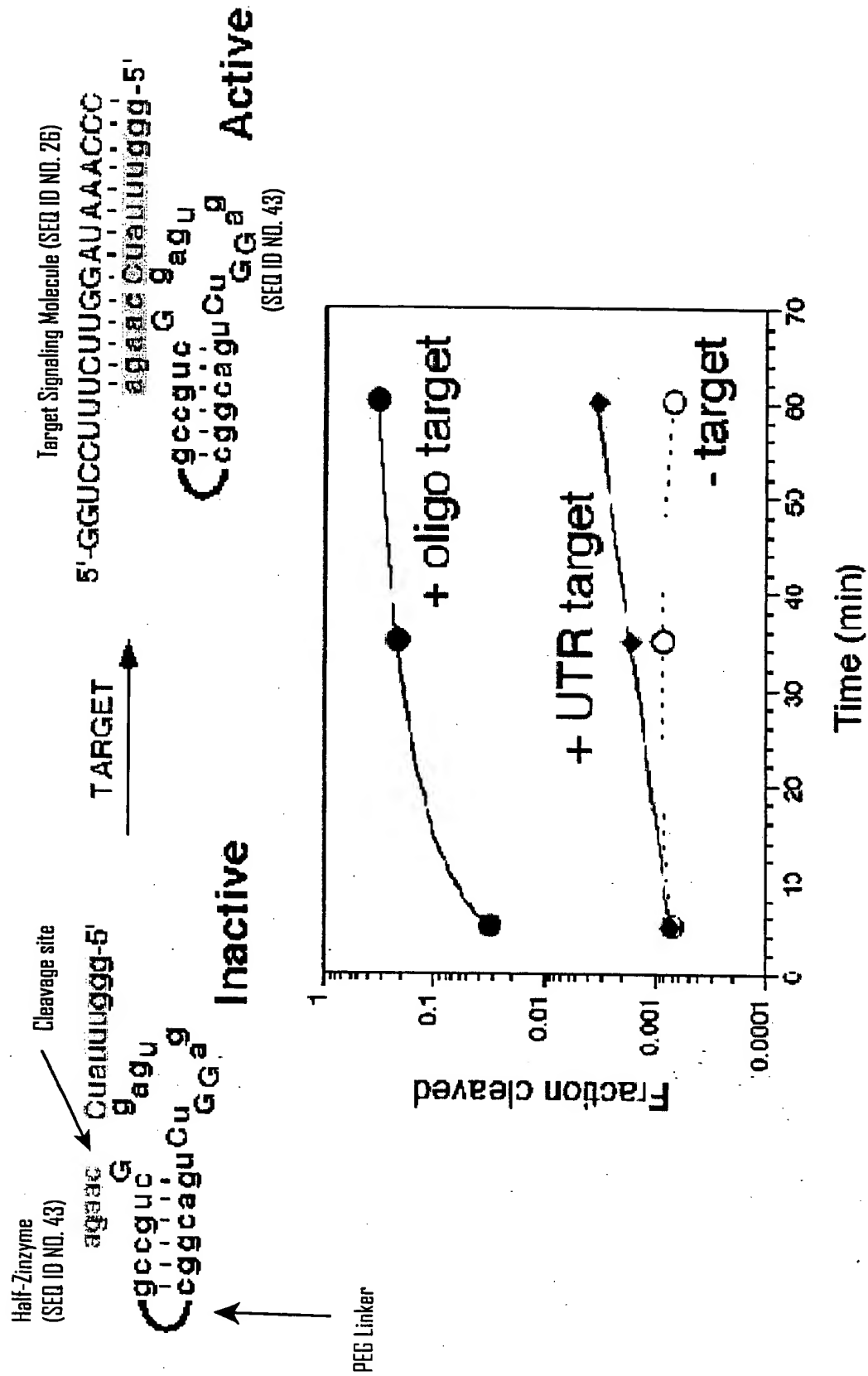


Figure 31

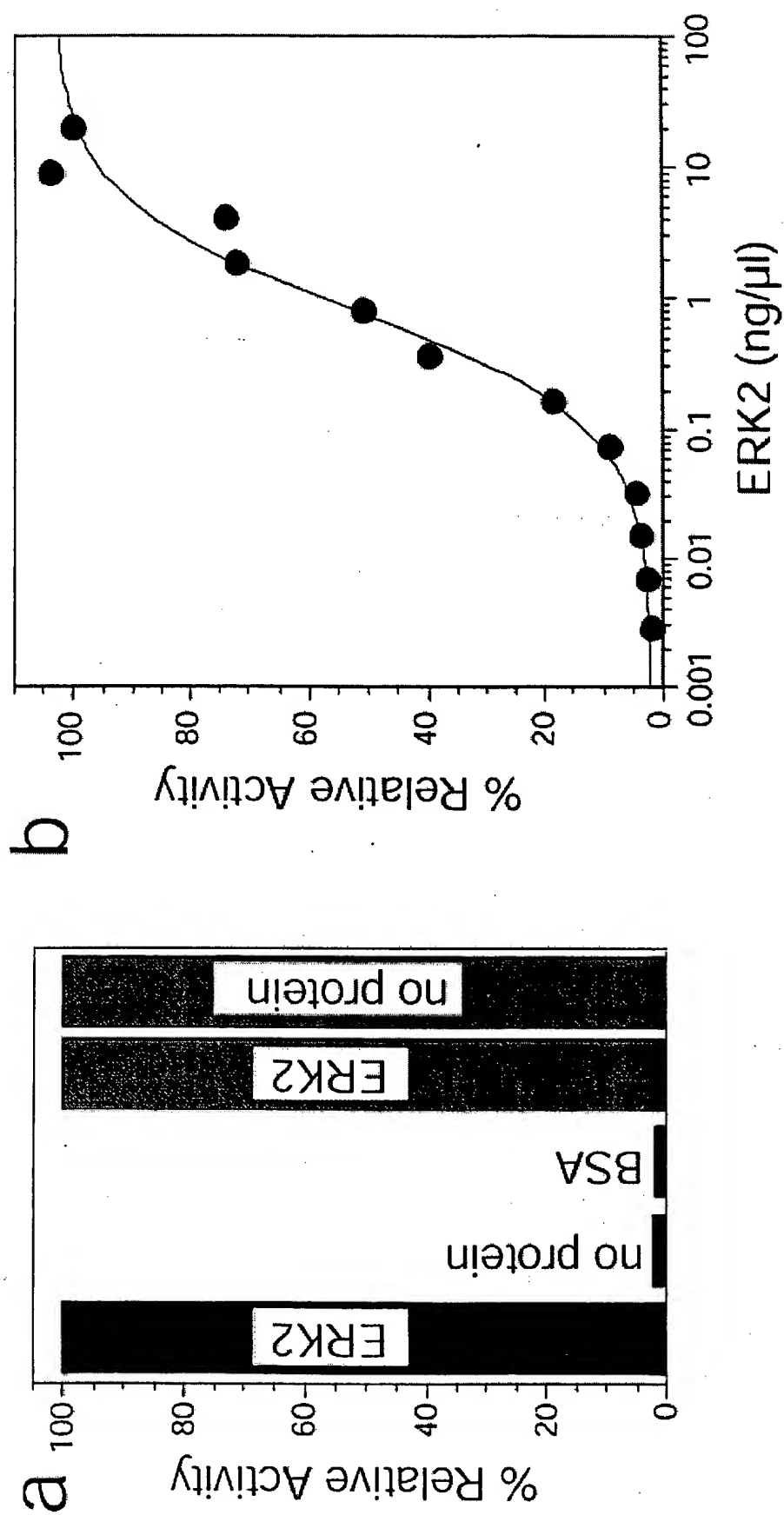
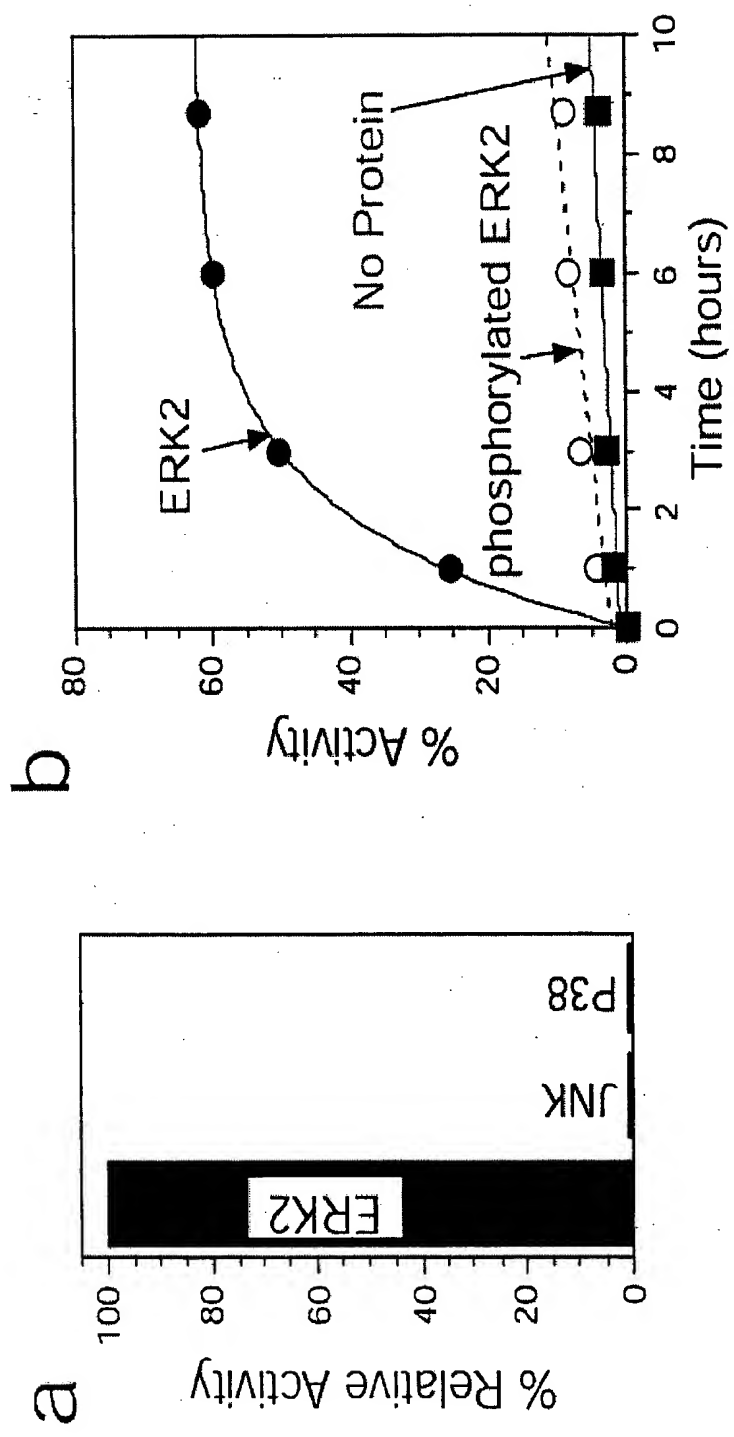


Figure 32



(SEQ ID NO: 47)

